THE INFLUENCE OF AGRICULTURAL SOIL PREPARATION METHODS ON THE PSEUDO-TOTAL ELEMENT CONTENT DETERMINED BY ICP-OES

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ABSTRACT. The five agricultural soil preparation methods for the purpose of determining the pseudo-total content of 20 elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si and Zn) in 4 soil samples taken from different plots of soils in the vicinity of Vranje (Southern Serbia) was applied in this paper. The following methods were used: digestion with aqua regia and hydrogen-peroxide with heating at temperature 190 ^{0}C, digestion with aqua regia and hydrogen-peroxide in combination with ultrasound with heating at temperature 95 ^{0}C, digestion with reverse aqua regia and hydrogen-peroxide with heating at temperature 190 ^{0}C, and digestion with nitric acid and hydrogen-peroxide under reflux with heating at temperature 95 ^{0}C, and digestion with perchloric acid with heating at temperature 190 ^{0}C. The results were statistically processed using an ANOVA test. The results obtained by this study show that the amount of extracted elements from the soil is influenced by several different factors including the following: soil type, soil content and chemical form of the elements.

Keywords: soil, digestion methods, macro and microelements, inductively coupled plasma optical emission spectrometry, ANOVA

INTRODUCTION

Soil, as the main habitat of humans, plants, and animals, is a highly specific component of the biosphere, which consists of mineral constituents, organic matter or humus, living organisms, air, water, and which regulates the natural cycles of these components. Soil acts as a natural buffer that

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controls the transport of chemical elements and substances into the atmosphere and hydrosphere, but it also represents a geochemical reservoir of contaminants. The most crucial role of soil is its productivity, which is responsible for the survival of mankind. Although the average content of elements in soil mostly stems from the parent material (rock), anthropogenic factors also contribute significantly to the total content in the soil [1,2].

Plants need elements for natural growth and development. Some elements present in larger amounts are harmful to the plant and they cause visible effects on it (leaf chlorosis, smaller growth, smaller yield). It is necessary to monitor soil contamination, especially that caused by heavy metals, in order to maintain a healthy environment. Heavy metals are naturally found in soils formed by the geological processes of change, erosion, and alteration. Heavy metals are not biodegradable, they accumulate in the environment and come from chemical and metallurgical industry, mining, traffic, domestic and commercial activities, waste incineration plants, industrial wastewater, sewage sludge, poor quality fertilizers, pesticides, fungicides, or as a result of anthropogenic activities in general. Deforestation, soil erosion, extraction processes from natural resources, climate change, precipitation, floods, as well as wet and dry atmospheric deposition also affect soil composition. The contents of Cd, Cr, Cu, Pb, and Zn are the best indicators of human influence. Mn is also known to easily migrate into the environment. The overuse of pesticides and fertilizers in agriculture causes the increase in the concentration of macro and microelements in agricultural soil. The presence of heavy metals in soil affects the quality of food and groundwater, microorganism activity, and plant development [3-7].

The total metal concentration in soil does not necessarily correspond to the amount of metals that are bioavailable to plants. Clay minerals, hydrated metal oxides (mostly Fe, Mn, and Al), as well as organic matter, are considered the most important components of soil, and it is exactly these components that dictate the bioavailability of elements. Moreover, physical, chemical, and biological characteristics of soil (soil texture, redox potential, pH, buffer capacity, cation exchange capacity, organic matter amount, clay amount, sulfate, carbonate, and hydroxide amount, soil humidity and temperature, and the microorganisms inhabiting it) also affect the migration and bioavailability of elements. In addition, the uptake of elements by plants is also affected by plant type, age, plant parts, growth period, element stability, and the element transfer factor [4,8,9]. The chemical form of the metals in soil can largely affect their behavior, toxicity, bioavailability, as well as the chemical reactions they participate in, which further influences the motion of this metal among the parts of the complex ecosystem (water, air, biota, and soil/sediments). Heavy metals can be retained by some soil components, so as to precipitate or coprecipitate as sulfides, carbonates, oxides, or hydroxides [10].

Soil sample preparation as an analytical step is very important and it often participates in the obtained results with the largest error, which is why the selection of sample preparation method is one of the most important factors that influence the validity of the obtained results. Several methods, more or less different, with different efficiency of so-called element extraction, different duration of the process and, most importantly, different requirements for laboratory equipment have been described in the literature. An important criterion when choosing the method is whether certain information about the total element concentration (*total content*), element concentration apart from the one mainly related to the silicate fraction (*pseudo-total content*) or about the amount of bioavailable elements is needed.

The efficiency of a soil preparation method depends on the nature of the element, matrix content, and decomposition conditions. During the selection of optimal soil preparation conditions, the mass of the sample, the composition of oxidizing agents and mixtures, the final volume, as well as the physical parameters (temperature, ultrasonic wave intensity, etc.) are varied. There are no universal methods, and the goal is to achieve extraction maximum, work minimum, agent minimum, and the highest environmental protection. The recovery values of pseudo-total and total digestion vary depending on the mineral content, origin of the soil, digestion method, and the tested element. A method is considered satisfactory if about 90% of the element gets extracted [5,11].

The so-called wet digestion is one of the most commonly used methods for determining the total and pseudo-total content. It enables high sample decomposition, i.e., the decomposition of organic matter by heating the samples in the presence of strong oxidizing agents (HNO_3, HCl, $HClO_4$, HF, H_2O_2, etc.) or their mixtures, where one agent serves for the decomposition and the other forms water-soluble complex salts with the elements that are being tested. Sulfuric acid is rarely used because of its construction of insoluble $PbSO_4$ and $BaSO_4$ and hydrofluoric acid due to its high aggressiveness. If the procedure is carried out in a closed system, the addition of HF degrades the silicate matrix, which leads to the so-called total digestion [12]. However, HF may also form insoluble precipitates with Al, Ca, Fe, and Mg, as well as coprecipitates with Rb, Sr, Y, Ba, Th, and U. Wet digestion can be carried out in open or closed vessels (autoclaves), using ultrasound or in microwave ovens. Wet digestion in open systems lasts pretty long and requires greater amounts of the solvent. It can also come to the loss of volatile analytes, and there is also a risk of soil contamination. Microwave digestion as a contemporary method of sample preparation, where the preparation time is shorter and the amount of the solvent is smaller, is commonly used for the

analysis of smaller amounts of analytes. The mixture of conc. HCl and conc. HNO_3 (3:1, *v/v*, so-called *aqua regia*) has been most commonly used [2,4,6,7,11-15]. This mixture is one of the most aggressive solvents with a greater sample decomposition effect compared to individual acids. What is varied is the amount of the mixture, time, temperature, as well as whether the procedure is carried out in an open or closed system. This is a very effective method for the extraction of heavy metal pseudo-total content in soil and it is most commonly used in the European Union. It is often used as a standard method for the preparation of reference soil samples in some countries (the UK and France). Nitric acid oxidizes hydrochloric acid producing various oxidating, highly reactive products, chlorine and nitrosyl-chloride. It dissolves even precious metals, but does not dissolve silicates completely. Nitrosyl-chloride dissolves sulfides, sulfosalts, selenides, tellurides, arsenides, while the present chlorides as a complexing reagent make this method highly effective.

In terms of other solvents, either individual or in a mixture, the following ones are used: aqua regia with the addition of $HClO_4$ [11,16]*;* aqua regia with the addition of H_2O_2 using microwave digestion [17]*;* the mixture of conc. HCl and conc. HNO_3 (1:3, *v/v*) in an open system [18]*;* the mixture of conc. HCl and conc. HNO_3 of different ratios in a microwave oven [11]*;* the mixture of conc. HCl, conc. HNO_3 and conc. HF (9:3:2, *v/v/v*) in a microwave oven [19]; dilute nitric acid (1:1*, v/v*) in an open system with the addition of H_2O_2 [11]*;* conc. nitric acid in a microwave oven (USEPA Method 3051A) [10,20]; boiled 2M HNO_3 [3]; nitric acid and hydrogen-peroxide with microwave digestion (USEPA Method 3050a) [21]; HNO_3 and HF in an oven (3:2, *v/v*) [5]; 20% nitric acid [6]; nitric and hydrofluoric acid [22]; nitric acid and hydrogen-peroxide [23,24].

Samples are often burned in an oven (the so-called dry process) with the aim of decomposing organic matter, after which the dry residue is treated with a solvent or solvent mixture [6]. The procedure of the so-called sequential extraction is also not uncommon: of exchangeable elements with magnesium-nitrate, carbonates with sodium-acetate, oxides with hydroxylamine and hydrochloric acid, organic matter with hydrogen-peroxide and residues with hydrogen-peroxide, hydrofluoric, nitric and perchloric acid [25].

There are a number of papers in the literature where different extraction procedures were carried out with the aim of assessing phytoavailable amounts i.e. the amounts of elements that are available to plants for adoption: Cd, Cu, Pb, and Zn with potassium-nitrate [15]; K, Ca, and Mg with ammonium-acetate; Fe, Zn, Mn, and Cu with DTPA [4]; K with ammonium-acetate; Zn, Cu, Cd, and Ni with DTPA [26]; Cu, Zn, and Cd with sodium-nitrate; P and K with water saturated with carbon-dioxide; Mg with calcium-chloride [3]; Na,

K, Ca, and Mg with ammonium-acetate [2]; K, Mg, and Na with ammonium-acetate, S with calcium-chloride, P with sodium-bicarbonate, Fe, Zn, Mn, and Cu with DTPA, B with hot water [27]; Ca and Mg with potassium-chloride; P, K, Fe, Zn, Mn, and Cu with a mixture of dilute HCl and H_2SO_4 [20]; different fractions of arsenic with ammonium-chloride, ammonium-fluoride, sodium-hydroxide, sulfuric acid [21]; Co, Cd, Cr, Pb, and Ni with ammonium-chloride, calcium-chloride, strontium-chloride, DTPA [10]; heavy metals with ammonium-acetate [6].

In this paper the efficiency of five accessible and most commonly used methods of soil sample preparation for determining the pseudo-total content of elements using inductively coupled plasma optical emission spectrometry (ICP-OES) was investigated.

RESULTS AND DISCUSSION

The validation process of the applied ICP-OES methods included the linearity of the calibration curve, the correlation coefficients, the limit of detection (LOD) as well as possible matrix interferences (Table 1). The calibration lines on several wavelengths were constructed for each element. The selection of the analytical emission line was made based on the calibration line parameters (maximum relative emission intensities, the correlation coefficients, the limit of detection), as well as based upon the tables of known interferences, baseline shifts and the background correction (the highest signal-to-background ratio) which was manually selected for the quantitative measurements.The limit of detection was calculated using the 3σ criterion [28]. The possible matrix interferences were examined by comparing the calibration slopes of the external calibration lines to the slope of the standard addition method lines [29]. The ratio $Slope_{cal}/Slope_{sam}$ values closer to 1 indicate a negligible matrix effect at the selected wavelengths that is the matrix has less effect on the quantitative determination of the elements. The obtained results for the ME are from -5.12 % to 1.21%, which indicates that the selected wavelengths can be used because they are at a satisfactory level of influence of the samples matrix.

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Table 1. The parameters of the selected analytical lines

Element	λ (nm)	LOD (ppm)	r	Linear range (ppm)	Ratio Slope$_{cal}$/Slope$_{sam}$	ME[*] (%)
Al	396.152	0.0077919	1	0-100	1.0065	0.65
As	189.042	0.0035719	1	0-1	0.9702	-2.98
Ca	396.847	0.0445667	0.9998	0-100	1.0057	0.57
Cd	226.502	0.0002784	1	0-1	1.0104	1.04
Co	228.616	0.0002645	1	0-1	0.9981	-0.19
Cr	267.716	0.0004410	1	0-1	1.0101	1.01
Cu	324.754	0.0003766	0.9999	0-1	1.0074	0.74
Fe	259.941	0.0028537	0.9999	0-100	1.0097	0.97
Hg	184.950	0.0001166	1	0-0,025	0.9919	-0.81
K	766.491	0.0122200	0.9999	0-100	0.9897	-1.03
Mg	279.553	0.0002343	1	0-10	0.9488	-5.12
Mn	257.611	0.0001789	0.9997	0-1	1.0011	0.11
Mo	202.095	0.0009466	0.9999	0-1	1.0121	1.21
Na	589.592	0.0070642	1	0-100	1.0100	1.00
Ni	231.604	0.0005709	0.9999	0-1	1.0074	0.74
P	177.495	0.0006585	0.9999	0-100	0.9864	-1.36
Pb	220.353	0.0088547	1	0-1	0.9955	-0.45
S	180.731	0.0088103	1	0-100	0.9973	-0.27
Si	251.612	0.0137430	0.9996	0-100	1.0067	0.67
Zn	206.200	0.0003018	0.9992	0-1	1.0093	0.93

[*]ME = $(\frac{I_1}{I_2} - 1) \times 100\%$;

I_1 – the intensity of the selected line in the standard solution;
I_2 – the intensity of the selected line spiked standard solution

The 5 methods listed above (M1-M5) were applied for the purpose of preparing the 4 agricultural soil samples (1-4). The obtained results concerning the content of the elements that were tested are given in Tables 2, 3, and 4.

Table 2. Mean content ± SD (mg kg^{-1}) of the elements extracted using different sample preparation methods

	Al	As	Cd	Ca	Cr	Co	Cu
1M1	8767±40	5.6±0.2[a]	1.15±0.02	2745±6	21.9±0.2[a]	11.0±0.2	18.8±0.1
1M2	6046±50	3.4±0.2	0.93±0.03[a,b]	2300±50	30.72±0.01	10.3±0.2	23.09±0.08
1M3	12849±40	5.5±0.2[a]	0.80±0.02	1975±30	21.85±0.06[a]	9.92±0.08	25.1±0.5
1M4	20306±40	6.58±0.08[b]	0.96±0.02[a,c]	2928±30	31.8±0.2	12.23±0.08[a]	37.8±0.2
1M5	13868±80	6.6±0.2[b]	0.95±0.02[b,c]	2612±9	27.12±0.03	12.18±0.06[a]	39.3±0.2
2M1	7194±30[a]	3.6±0.1[b]	0.81±0.03	2050±8[a]	14.65±0.08	8.2±0.2[a]	17.6±0.2
2M2	9751±30	5.0±0.2	0.55±0.01[a]	2023±20[a]	15.81±0.08	7.76±0.08	25.1±0.3
2M3	6748±30	3.6±0.2[b]	0.49±0.02	1684±20	11.2±0.2	6.20±0.08	23.3±0.2
2M4	11329±40	4.2±0.2[a]	0.56±0.02[a]	2377±20	16.67±0.08	8.24±0.06[a]	36.7±0.2
2M5	7266±40[a]	4.5±0.2[a]	0.62±0.02	2127±20	13.97±0.08	7.2±0.2	28.1±0.2
3M1	10712±50[b]	4.32±0.06[a,c]	1.38±0.04	3030±5	24.58±0.09	11.01±0.08	29.9±0.3
3M2	10711±40[b]	4.8±0.2	0.563±0.006[a,b]	2119±20[a]	20.36±0.08[a]	8.86±0.03	38.7±0.2
3M3	8872±30[a]	3.8±0.2[b,e]	0.60±0.02[a,c]	1810±20	16.05±0.03	7.22±0.03	34.8±0.3
3M4	12434±50	4.1±0.2[b,c,d]	0.59±0.02[b,c]	2087±20[a]	19.73±0.03	8.1±0.2	45.2±0.3
3M5	8802±40[a]	3.9±0.3[a,d,e]	1.28±0.03	2398±6	20.4±0.02[a]	9.2±0.2	46.3±0.3
4M1	8474±40	4.71±0.08[a,c]	0.87±0.02[c]	7749±40	16.26±0.08[c,d,e]	8.5±0.08	22.54±0.08
4M2	12933±40	6.5±0.2	0.61±0.02[a]	12258±40	17.1±0.2[a,b,d]	8.2±0.2	35.4±0.2[a]
4M3	7905±40	5.1±0.2	0.642±0.008[a,b]	8833±30	11.71±0.08	7.04±0.06	29.17±0.06
4M4	11081±60	4.8±0.2[b,c]	0.66±0.02[b]	12867±40	15.64±0.08[b,e,f]	7.87±0.08	89±3
4M5	6594±50	5.0±0.2[a,b]	0.88±0.03[c]	12415±60	15±3[a,c,f]	7.52±0.03	36.6±0.3[a]

*the amounts of an element marked with the same letters within one and the same sample (by column) do not significantly differ statistically; a is always less than b; b is always less than c, etc.; the greater the value of the letters a, b, c, etc. the less the obtained results differ

Table 3. Mean content ± SD (mg kg^{-1}) of the elements extracted using different sample preparation methods

	Fe	Pb	Mg	Mn	Ni	K	Na
1M1	28747±60	12.3±0.2[c]	4067±20	592±2[a,b]	20.5±0.1	4375±8	555±2
1M2	32552±80	12.3±0.4[b,c]	4844±3	601±4[a]	26.1±0.2[a]	6632±30[a]	88±2
1M3	30736±90	12.71±0.08[b]	5289±9	584±7[b]	26.0±0.3[a]	6154±20	134±2
1M4	35921±50	17.9±0.2[a]	6248±30	675±4	26.7±0.2	7431±20	172.2±0.8
1M5	33868±50	18.25±0.08[a]	5832±20	646±2	29.1±0.2	6643±50[a]	145±2
2M1	13514±70[a]	15.7±0.2	2297±5[a]	506±2[b]	13.3±0.1[a]	2693±8[a]	530±4
2M2	13351±30	17.4±0.2	4000±20	531±4[a]	20.3±0.4	3095±30	82±2
2M3	11370±40	18.1±0.2	2049±20	505±5[b]	13.2±0.3[a]	3018±30	130±2
2M4	13589±70[a]	22.6±0.1	2444±30	597±2	19.2±0.5	3321±20	226±2
2M5	12584±80	19.9±0.2	2261±8[a]	528±2[a]	17.5±0.3	2660±7[a]	121±2
3M1	30594±70	8.5±0.2[a]	4539±7	557±2	20.8±0.2	4799±6	479±2
3M2	24688±80	9.4±0.2[b]	4052±20	461±3	24.4±0.1	4421±9	82±3
3M3	23947±90	8.71±0.08[a]	3917±30	467.5±0.9	19.4±0.3	3529±9	95±2
3M4	25184±60	9.4±0.2[b]	4159±10	495.5±0.8[a]	18.8±0.2	4165±10	123±1
3M5	25500±60	11.8±0.2	3766±4	495±2[a]	17.96±0.08	4038±5	145.4±0.8
4M1	13045±80[a]	19.9±0.4	2704±5[a]	552±2	15.13±0.08[a]	2613±1	864±2
4M2	15455±60	22.04±0.05	3320±30	640±3	18.0±0.2	3172±20	228±2[a]
4M3	12568±80	24.7±0.4	2662±30[a]	622±4	14.8±0.2[a]	2222±10	184±3
4M4	14329±70	28.5±0.2	2931±40	631±2	23.4±0.2	2864±20	269±2
4M5	12895±60[a]	20.7±0.2	2360±8	581±2	13.68±0.05	2135±6	226.3±0.8[a]

*the amounts of an element marked with the same letters within one and the same sample (by column) do not significantly differ statistically; a is always less than b; b is always less than c, etc.; the greater the value of the letters a, b, c, etc. the less the obtained results differ

The obtained results were statistically processed using the *one-dimensional variance analysis* method (*One-way ANOVA*) with the application of the *Tukey post-hoc test* for the purpose of finding the contents which do not exhibit a statistically significant difference, i.e., for the purpose of establishing for which element(s) and in which sample(s) the different soil preparation methods do not statistically differ. Based on the obtained results, in general, it can be concluded that there are no regularities neither per sample nor per

the methods applied or the element tested, which suggests that the efficiency of the element extraction with the application of these methods is affected by a number of different factors. It is evident that the smallest statistically significant difference, as a result of the application of different methods, can be found in the contents of the following elements: As, Cd, Pb, Cr, Mn and Ni, while most of the statistically significant differences can be found among the contents of macroelements.

Table 4. Mean content ± SD (mg kg^{-1}) of the elements extracted using different sample preparation methods

	Zn	Mo	P	Si	S	Hg
1M1	95.7±0.3	0.49±0.03[a]	727±2[a]	140±2	326±2[a]	0.0574±0.0003
1M2	100.4±0.5[a]	3.30±0.05	726±4[a]	422±4	461.2±0.8	0.3286±0.0004
1M3	100.7±0.3[a]	<LOD	623±3	14.7±0.5	312±2	0.0651±0.0006
1M4	158.6±0.3	0.53±0.02[a]	781±3	66±2	329±3[a]	0.0333±0.0002
1M5	155.4±0.6	0.78±0.02	812±3	440±2	381±2	0.1214±0.0006
2M1	89.7±0.6	0.37±0.03	797±2	96±2	299±2	0.0522±0.0003
2M2	96.1±0.3	0.48±0.03	959±3	538±3	384±2	0.0505±0.0001
2M3	92.1±0.3	<LOD	785±5	19.6±0.4	324±2	0.0287±0.0002
2M4	150.0±0.3	1.942±0.008	885±3	49.2±0.3	367±2[a]	0.0374±0.0002
2M5	115.6±0.3	<LOD	904±3	555±2	363±3[a]	0.1227±0.0005
3M1	113.6±0.3	0.39±0.02	760±2	67±1	296±2	0.0766±0.0001
3M2	80.4±0.3	0.51±0.02[a]	730±4	297±2	209±2[a]	0.1162±0.0002
3M3	84.3±0.5	0.30±0.02	592±3	14.4±0.2	227±3	0.0268±0.0003
3M4	107.3±0.3	0.48±0.04[a]	626±3	79±2	207±2[a]	0.0313±0.0004
3M5	132.2±0.3	<LOD	696±1	409.9±0.8	337±2	0.189±0.002
4M1	102.5±0.6	0.40±0.03	2113±4	53.1±0.8	596±2	0.0818±0.0003
4M2	130.3±0.3	0.78±0.02	2821±3	491±3	826±2	0.0224±0.0002
4M3	112.6±0.6	<LOD	2384±3	17.6±0.4	651.2±0.8	0.0275±0.0002
4M4	321.4±0.3	2.01±0.02	2584±4[a]	151.1±0.8	787±3	0.0520±0.0003
4M5	147.4±0.3	0.62±0.01	2586±2[a]	641±2	697±2	0.0747±0.0001

*the amounts of an element marked with the same letters within one and the same sample (by column) do not significantly differ statistically; a is always less than b; b is always less than c, etc.; the greater the value of the letters a, b, c, etc. the less the obtained results differ

In order to compare the efficiency of the applied methods more easily, relative content deviations in those samples where the M2, M3, M4, and M5 methods were applied in relation to the samples where the M1 method was applied are given in Tables 5 and 6:

$$rel.deviation = \frac{X_{MN} - X_{M1}}{X_{M1}} \times 100\% \qquad (1)$$

where X_{M1} is the mean element content (mg kg^{-1}) in the sample prepared using method M1; X_{MN} is the mean element content (mg kg^{-1}) in the sample prepared using methods M2, M3, M4, and M5.

Table 5. Relative deviation (%) of the content of the tested elements in the samples prepared using methods M2, M3, M4, and M5 in relation to method M1

Element	sample-1				sample-2			
	M2	M3	M4	M5	M2	M3	M4	M5
Al	-31.03	46.56	131.62	58.18	35.55	-6.20	57.49	1.00
As	-39.29	-1.79	17.50	18.23	38.09	-1.17	16.87	25.66
Cd	-18.73	-30.08	-16.37	-17.34	-32.88	-39.26	-30.58	-24.23
Ca	-16.22	-28.06	6.66	-4.83	-1.29	-17.87	15.94	3.75
Cr	39.97	-0.43	44.97	23.57	7.93	-23.87	13.79	-4.63
Co	-6.06	-9.89	11.08	10.65	-5.18	-24.24	0.76	-12.26
Cu	22.62	33.18	100.88	108.94	43.13	32.76	109.16	59.86
Fe	13.23	6.92	24.96	17.81	-1.21	-15.87	0.55	-6.89
Pb	0.53	3.61	45.65	48.73	10.58	15.43	43.97	26.28
Mg	19.12	30.06	53.64	43.41	74.10	-10.82	6.40	-1.58
Mn	1.45	-1.29	13.97	9.17	4.92	-0.24	18.02	4.26
Ni	27.22	26.77	30.13	42.08	52.89	-0.32	44.67	31.76
K	51.59	40.66	69.84	51.84	14.91	12.05	23.32	-1.25
Na	-84.11	-75.78	-68.98	-73.96	-84.57	-75.53	-57.29	-77.23
Zn	4.86	5.16	65.63	62.31	7.11	2.71	67.25	28.83
Mo	576.38	/	8.58	58.92	31.25	/	431.37	/
P	-0.17	-14.40	7.41	11.61	20.43	-1.51	11.06	13.52
Si	202.23	-89.45	-53.02	214.77	461.77	-79.50	-48.69	479.72
S	41.53	-4.14	0.91	17.02	28.72	8.67	22.86	21.58
Hg	472.69	13.44	-42.00	111.53	-3.14	-45.06	-28.38	135.08

The positive and negative deviation values in the tables express the percentage of how much more or less of an element was extracted using the given method (M2-M5) in relation to the method M1. The analysis of the obtained results shows that the greatest extraction efficiency is achieved by pseudo-total extraction with nitric acid under reflux (M4) for the largest number of the elements tested in sample 1 (Al, Ca, Cr, Co, Fe, Mg, Mn, K, and Zn). In terms of efficiency, the method with aqua regia using ultrasound (M5) follows, which is mostly used for the extraction of As, Cu, Pb, Ni, P, and Si. Better contact between the sample and the reagent applied using reflux and ultrasound must have contributed to that. In terms of sample 2, the highest efficiency was also noticed in the case of the M4 method applied for the following elements: Al, Ca, Cr, Co, Cu, Fe, Pb, Mn, K, Zn, and Mo. Then follows the M2 method with aqua regia for As, Mg, Ni, P, Si, and S. Sample 3 was specific in that the reverse aqua regia method proved most effective for Cd, Ca, Cr, Co, Fe, Mg, Mn, K, Na, and P. This method turned out to be the best for Cd and Na in samples 1 and 2, as well as for Na, Co, and Hg in sample 4. Then follows the M5 method for Cu, Pb, Zn, Si, S, and Hg. The greatest variability in method efficiency was noticed in sample 4 where two of the methods stood out: the M2 method proved best for the extraction of the following elements: Al, As, Cr, Fe, Mg, Mn, K, P, and S, while the M4 method proved most favorable for the following elements: Ca, Cu, Pb, Ni, Zn, and Mo.

Even with these variabilities observed, some general conclusions can be drawn. It is characteristic for Na that the M1 method was best for all of the examined samples, while the results of the analyses concerning Si showed that the most favorable method was M5, where aqua regia was used as a very aggressive and destructive reagent with the application of ultrasound. Comparing the concentration values for Si obtained using methods M2 and M5 which utilize aqua regia as a reagent, it can be observed that the greatest amounts of this mineral were extracted by these methods, with the concentration values obtained using ultrasound which provided a better phase contact being slightly higher. It is widely known that mineral acids like HCl, HNO_3, and $HClO_4$, which are generally used for element extraction both individually and in combination with one another, do not dissolve the silicate fraction of the sample, or dissolve it very little. During the experimental execution of soil preparation after the digestion had been conducted, a certain amount of silicate fraction remained, which is why the amount of Si in the tested samples does not represent the total content of this mineral. Al, Cd, Ca, Cu, Fe, Pb, and Zn were best extracted by method M4 in most of the samples, As and S by method M2, and Hg by method M5. Similar behavior in terms of for which sample which method proved best was observed in the following groups of elements: Cr, Co, Mn, and K; Cu, Pb, and Zn; Ca and Fe, which suggests the same chemical form in which these elements are present in the tested samples.

Table 6. Relative deviation (%) of the content of the tested elements in the samples prepared using methods M2, M3, M4, and M5 in relation to method M1

Element	sample-3				sample-4			
	M2	M3	M4	M5	M2	M3	M4	M5
Al	-0.01	-17.17	16.08	-17.84	52.62	-6.71	30.77	-22.18
As	11.57	-12.22	-5.14	-8.87	38.30	9.15	1.06	5.63
Cd	-59.07	-56.39	-56.79	-7.32	-29.48	-25.93	-23.61	0.95
Ca	-30.06	-40.25	-31.13	-20.86	58.19	13.98	66.04	60.21
Cr	-17.17	-34.71	-19.75	-16.86	5.33	-27.96	-3.79	-6.78
Co	-19.56	-34.47	-26.48	-16.58	-3.23	-17.07	-7.25	-11.38
Cu	29.52	16.41	51.34	54.81	56.93	29.41	292.82	62.56
Fe	-19.30	-21.73	-17.68	-16.65	18.48	-3.66	9.85	-1.14
Pb	10.19	2.62	10.46	38.91	10.87	24.03	43.31	4.03
Mg	-10.73	-13.70	-8.38	-17.03	22.78	-1.54	8.42	-12.70
Mn	-17.13	-16.04	-11.01	-11.17	15.91	12.66	14.31	5.30
Ni	17.42	-6.46	-9.66	-13.51	19.14	-2.24	54.53	-9.61
K	-7.87	-26.45	-13.21	-15.84	21.39	-14.99	9.61	-18.29
Na	-82.93	-80.22	-74.24	-69.66	-73.63	-78.69	-68.84	-73.80
Zn	-29.20	-25.79	-5.56	16.40	27.14	9.92	213.59	43.86
Mo	28.96	-24.27	22.91	/	95.63	/	401.00	55.88
P	-3.90	-22.10	-17.59	-8.38	33.50	12.79	22.26	22.37
Si	341.66	-78.56	18.31	510.17	824.73	-66.82	184.32	1105.60
S	-29.62	-23.52	-30.02	13.79	38.51	9.22	31.96	16.84
Hg	51.70	-65.04	-59.13	147.13	-72.59	-66.40	-36.41	-8.65

Based on the presented results, it is evident that the pseudo-total element extraction using concentrated perchloric acid did not prove most efficient for any element in any sample, which is why this method is not suitable for the preparation of these types of soil. Another reason for this is that hydrogen-peroxide, which is normally recommended for the additional decomposition of the present organic matter, was not used in this method.

Apart from the papers cited in the introduction in which different soil preparation methods were used, the results concerning the efficiency of different methods are also present in the literature. For instance, *Safarova et al.* [11] tested the extraction efficiency of Cd, Pb, Co, Ni, Zn, Mn, Cu, and Hg from solid samples (soil, rocks, sediments, and industrial waste) using the different methods. The obtained results showed that the different efficiency of the applied methods depended on the applied soil sample. Based on the

results obtained from the study, the authors concluded that there was no universal method for the decomposition of different samples and that the selection of the preparation method depended on the nature of the element, composition of the sample or matrix, decomposition conditions, equipment availability, and the urgency of the analysis. They also noticed that there were no significant differences between the open system decomposition and microwave digestion for the following elements: Pb, Cu, Fe, Zn, and Mn as well as the microwave digestion and the digestion in an autoclave were the methods of the highest efficiency. *Santos and Alleoni* [12] applied the EPA 3051 method and the aqua regia method to determine the pseudo-total concentration of Cd, Co, Cr, Cu, Ni, Pb, and Zn in soil samples. Based on the results obtained, they concluded that the aqua regia method was far more efficient than the USEPA 3051 method in terms of the amount of Co, Ni, Pb, and Zn extracted, while these two methods did not differ significantly concerning the extraction of Cr and Cu. *Oliviera et al.* [30] concluded that the extraction with aqua regia was a satisfactory method for the purpose of assessing the phytoavailable amounts of elements, as well as the elements that can be excreted into natural waters. *Florian* [31] noticed that the aqua regia method was more suitable for the digestion of samples when it comes to the determination of trace elements which are potentially hazardous for the environment. Also, the comparison of 3050A and 3050BA methods showed that the 3050B method resulted in a better recovery of Ag, As, Ba, Be, Co, Cu, Sb, and V, and that the 3050A method resulted in a better recovery of Cd, Cr, Mo, Ni, Pb, Sb, and Zn. Also, there was no difference between the microwave digestion and the digestion on a heating surface for Cu, Pb, Zn, Cr, and Ni [23].

The observed variability concerning the efficiency of different methods in this paper and in the papers of other authors might be the result of a number of different factors: soil type, quantitative and qualitative soil composition (metals, non-metals, and organic matter), pH value of soil solution, electrical conductivity, chemical form in which the elements are (oxides, sulfates, carbonates, sulfides, complexes etc.).

CONCLUSION

The results concerning the determination of the tested elements' content in agricultural soils prepared by different methods have shown that there is no universal method, i.e., that the efficiency of the element extraction depends on its nature, on the chemical form in which it occurs, on the

composition of the soil i.e., soil type, etc. In most cases, the method with nitric acid and reflux and with aqua regia and ultrasound have shown the highest efficiency, while the method with perchloric acid has shown the lowest efficiency. It is also evident that the smallest statistically significant difference, as a result of the application of different methods, can be found in the contents of the following elements: As, Cd, Pb, Cr, Mn and Ni, while most of the statistically significant differences can be found among the contents of macroelements.

MATERIALS AND METHODS

Samples and reagents

In the fall of 2018, four soil samples were taken from different agricultural plots of soil near Vranje and each of them was prepared using different soil preparation methods for the purpose of assessing the extraction efficiency of the elements that were tested.

A shovel was used for soil sampling and the samples were taken at a depth of 15 to 20 cm from the surface of around 100 cm^2. A representative sample was made from each of the four agricultural plots of soil by taking a sample from five different places along the length of the plot and those samples were later homogenized, sifted through a sieve, and stored in polyethylene bags. Just before preparation, the samples were dried in the oven at 95 ^0C to constant weight. The dried samples were laid aside in desiccators to cool, after which approximately 1 g of each sample was measured.

The following chemicals were used for the preparation of soil samples: 65% HNO_3 (Sigma-Aldrich, SAD); 37% HCl (Sigma-Aldrich, SAD); 30% H_2O_2 (Macron, SAD); 70% $HClO_4$ (Sigma-Aldrich, USA). The following standards were used for the construction of a calibration line: multielement standard ULTRA SCIENTIFIC #2: Al, As, Ba, Be, Bi, Cd, Ca, Ce, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mg, Mn, Ni, K, Rb, Se, Na, Sr, Tl, U, V, and Zn (10 µg cm^{-3}), multielement standard ULTRA SCIENTIFIC #4: B, Ge, Mo, Nb, P, Re, Si, S, Ta, Ti, W, and Zr (10 µg cm^{-3}) (USA). 99.996% purity argon was used for the operation of ICP-OES. Deionized water ($ϰ$=0.05 µS cm^{-1}) was used for the purposes of preparing the solutions and washing the laboratory dishes.

Instruments

Inductively coupled plasma optical emission spectrometer ICP-OES (Arcos Spectro, Germany); analytical balance (METTLER TOLEDO, Switzerland); ultrasonic bath (Bandelin SONOREX Digital 10 P, Sigma, USA); drying oven (LLG LABWARE uniOVEN 150, Germany); deionizator TKA MicroMed Wasseraufbereintungssysteme GmbH, Germany); stirrers (LLG LABWARE uniSTIRRER, Germany); automatic variable pipette (Transferpette S (0.5-5 cm^3), Germany).

The following operating conditions of the ICP-OES instrument were used: analysis pump rate 2 mL min^{-1}, RF power 1300 W, nebulizer gas flow rate 0.89 L/min, coolant gas flow rate 12 L min^{-1}, auxiliary gas flow rate 0.8 L min^{-1}, axial and radial plasma view mode.

Methods of pseudo-total metal extraction from soil

Pseudo-total metal extraction from soil using reverse aqua regia (M1)

Approximately 1 g of each sample was measured and moved to an Erlenmeyer flask. 12 cm^3 of conc. HNO_3 and 4 cm^3 of conc. HCl (HNO_3:HCl = 3:1, *v/v*) were added. The contents of the flasks were heated in a sand bath at a temperature of about 190 ^{0}C. In the end, 2 cm^3 of 30% H_2O_2 were added to each flask, after which the contents were cooled and filtrated using filter paper (black ribbon). The filtrate was collected in normal 50 cm^3 vessels [18,23].

Pseudo-total metal extraction from soil using aqua regia (M2)

Approximately 1 g of each sample was measured and moved to an Erlenmeyer flask. 12 cm^3 of conc. HCl and 4 cm^3 of conc. HNO_3 were added to each Erlenmeyer flask. The contents of the flasks were heated in a sand bath at a temperature of about 190 ^{0}C for an hour, after which 2 cm^3 of 30% H_2O_2 was added and heated until the foaming stopped. The mixture was cooled and filtrated through filter paper (black ribbon), while the filtrate was collected in a normal 50 cm^3 vessel [17].

Pseudo-total metal extraction from soil using concentrated perchloric acid (M3)

Approximately 1 g of each sample was measured and moved to an Erlenmeyer flask. 18 cm^3 of conc. perchloric acid were poured over the soil, after which the content was heated at a temperature of 190 ^{0}C for an hour. The mixture was cooled and filtrated through filter paper (black ribbon), and the filtrate was collected in a normal 50 cm^3 vessel [11].

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Pseudo-total metal extraction from soil under reflux (M4)

Approximately 1 g of each sample was measured and moved to a round-bottom flask. Then, 10 cm^3 of 1:1 (*v/v*) of nitric acid were added and magnet and reflux condenser were put. The mixtures were refluxed for 10 – 15 minutes at a temperature of 95 ^0C (without boiling) with stirring. The mixture was partially cooled, after which 5 cm^3 of conc. HNO_3 were added to it, the reflux condenser was placed again, and the mixture was refluxed for another 30 minutes. The final step was repeated one more time to ensure better oxidation of the sample. The mixture was then evaporated to a small volume (approximately 5 cm^3) without boiling (95 ^0C) and the reflux condenser was taken out. The mixture was cooled, after which 2 cm^3 of cold deionized water and 3 cm^3 of 30% hydrogen peroxide were added to it for the total oxidation of the sample. The flask containing the mixture was carefully heated with stirring but without the reflux condenser, while special attention was paid not to lose the mixture during its foaming. After the foaming had stopped, another 1 cm^3 of 30% hydrogen peroxide was carefully added to the mixture, and the procedure was repeated until the foaming caused by the recent addition of hydrogen peroxide stopped. The cooled mixture was then filtrated using filter paper (black ribbon), and the filtrate was collected in a normal 50 cm^3 vessel [23].

Pseudo-total metal extraction from soil using aqua regia in combination with ultrasound (M5)

Approximately 1 g of each sample was measured and moved to an Erlenmeyer flask. 12 cm^3 of conc. HCl and 4 cm^3 of conc. HNO_3 were poured over the measured soil. The Erlenmeyer flask was put inside an ultrasonic bath which had previously been heated to the temperature of 95 ^0C. It took an hour for the process of extraction, after which the sample was cooled. 3 cm^3 of 30% hydrogen peroxide were added to the cooled sample. In order to ensure the total oxidation, another 1 cm^3 of 30% hydrogen peroxide was added, and the procedure was repeated until the foaming caused by the recent addition of hydrogen peroxide stopped. It is important to avoid losing the mixture during its foaming, which is why the reaction mixture was heated gradually and carefully. The cooled mixture was then filtrated using filter paper (black ribbon) and the filtrate was collected in a normal 50 cm^3 vessel [11].

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